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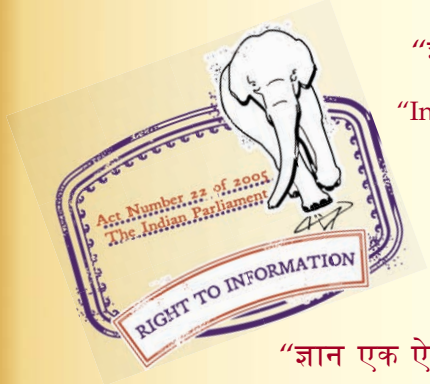

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Indian Standard

METHODS OF TEST FOR EDIBLE STARCHES AND STARCH PRODUCTS

PART II CHEMICAL METHODS

(First Revision)

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Indian Standard

METHODS OF TEST FOR EDIBLE STARCHES AND STARCH PRODUCTS

PART II CHEMICAL METHODS

(First Revision)

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Indian Standard

METHODS OF TEST FOR EDIBLE STARCHES AND STARCH PRODUCTS

PART II CHEMICAL METHODS

(First Revision)

0. FOREWORD

0.1 This Indian Standard (Part II) (First Revision) was adopted by the Indian Standards Institution on 28 June 1978, after the draft finalized by the Edible Starches and Starch Products Sectional Committee had been approved by the Agricultural and Food Products Division Council.

0.2 This standard was first published in 1968 to cover comprehensively the methods of test for edible starches. This revision has been taken up to up-date this standard by incorporating other methods employed for quality characteristic determination and by expanding its scope to cover starch products as well. It is being published in two parts. This standard (Part II) covers chemical methods while Part I covers physical methods.

0.3 It is expected that the adoption of this standard in India would help in defining the quality of edible starches and starch products in a manner that would enable better quality control. Besides, it will help in achieving uniformity in the methods of test of edible starches, thereby facilitating the interpretation and comparison of results.

0.4 In the preparation of this standard, due consideration has been given to the existing methods of test followed by the edible starch industry in the country. However, wherever possible, alignment has also been made with the final draft proposals formulated by the Technical Committee ISO/TC 93 Starch (Including Derivatives and By-Products) of International Organization for Standardization.

0.5 In reporting the result of a test or analysis made in accordance with this standard, if the final value, observed or calculated, is to be rounded off, it shall be done in accordance with IS: 2-1960*.

*Rules for rounding off numerical values (*revised*).

1. SCOPE

1.1 This standard (Part II) prescribes the chemical methods commonly used for testing of edible starches and starch products.

2. QUALITY OF REAGENTS

2.1 Unless specified otherwise, pure chemicals and distilled water (*see* IS : 1070-1977*) shall be employed in tests.

NOTE — 'Pure chemicals' shall mean chemicals that do not contain impurities which affect the result of analysis.

3. PREPARATION OF SAMPLE FOR TEST

3.1 For pure starches, take the starch powder as required for different tests. For starch products, take about 100 g of the material and grind it coarsely in a pestle and mortar for chemical analysis.

4. DETERMINATION OF MOISTURE

4.0 Two methods for the determination of moisture content in starch and starch product have been specified. Method I is to be used as a routine method while Method II is to be used as a reference method.

4.1 Method I

4.1.1 *Principle* — The method is based on the dehydration of the sample in a hot-air oven at a temperature of 105 to 110°C for a period of 4 hours.

4.1.2 *Procedure* — Dry a wide-mouthed glass weighing bottle in a drying oven at 100 to 105°C, cool in a desiccator, allow it to attain room temperature and weigh accurately. Weigh accurately 20 g of the test sample in the tared weighing bottle and note the mass. Place the weighing bottle with the sample in the drying oven, partly remove the stopper and dry the sample at 105 to 110°C to constant mass. (About 4 hours drying is sufficient.) Note the mass accurately. The sample shall be taken to have attained constant mass when two consecutive weighings taken at an interval of 30 minutes of drying do not differ by more than 2 mg. Preserve the dried material for the determination of total ash (**5**).

4.1.3 *Calculation*

$$\text{Moisture content, percent by mass} = \frac{100\ (M_1 - M_2)}{M_1}$$

where

M_1 = mass in g of the sample before drying, and

M_2 = mass in g of the sample on drying to constant mass.

*Specification for water for general laboratory use (*second revision*).

4.2 Method II (Oven Drying Method)

4.2.1 *Principle* — The method is based on the dehydration of the test portion in an electrically heated drying oven at a temperature of 130 to 133°C at atmospheric pressure for a period of 1 hour 30 minutes.

4.2.2 *Apparatus*

4.2.2.1 *Metal dish* — Unaffected by starch under the conditions of test, for example, aluminium, and having a suitable tight-fitting lid, suitable dimensions are 55 to 65 mm diameter, 15 to 30 mm height and about 0·5 mm wall thickness.

4.2.2.2 *Oven* — Constant-temperature, electrically heated and with a suitable air circulation.

4.2.3 *Procedure* — Carry out weighing to the nearest 0·001.

4.2.3.1 *Test sample* — Weigh the dish (**4.2.2.1**) and its lid after drying at 130°C and cooling in the desiccator. Transfer 5 ± 0·25 g of the well-mixed sample, which shall be free from any hard and lumpy material, to the dish with the minimum exposure to the atmosphere. Replace the lid and weigh immediately to determine the mass of the test sample. Distribute the test portion in a uniform layer over the bottom of the dish.

4.2.3.2 *Determination* — Place the open dish containing the test sample in the drying oven (**4.2.2.2**) preheated to 130°C, allowing the lid to lean against the dish, and dry at 130 to 133°C for 1 hour 30 minutes reckoned from the moment when the oven temperature again reaches 130°C. After this period, rapidly cover the dish and put it in the desiccator. The dishes should never be superimposed in the desiccator. Allow the test sample to cool to room temperature in the desiccator for 30 to 45 minutes. When the dish has cooled to room temperature, weigh it within 2 minutes of its removal from the desiccator. Carry out at least two determinations on the same well-mixed laboratory sample.

4.2.4 *Calculation*

$$\text{Moisture content, percent by mass} = (M_1 - M_2) \times \frac{100}{M_1 - M_0}$$

where

M_1 = mass in g of the dish with sample and lid before drying,

M_2 = mass in g of the dish with sample and lid after drying, and

M_0 = mass in g of the dried empty dish and lid.

NOTE — Take as the result the arithmetic mean of the two determinations, if the requirements concerning repeatability are satisfied. Report the result to the first decimal place.

4.2.5 *Repeatability* — The difference between the results of two determinations, carried out simultaneously or in rapid succession by the same analyst, shall not exceed 0·2 g in 100 g of the product. If it exceeds 0·2 g, the determination shall be repeated in duplicate after ensuring that the original sample is thoroughly mixed. If desired, a duplicate test should be made on another day by another analyst or in another oven. For the calculation, use only duplicate results that agree to within 0·2 percent in absolute value.

5. DETERMINATION OF TOTAL ASH

5.1 Weigh accurately 5 to 10 g of the material in a platinum, porcelain or silica dish. Ignite the material in the dish with the flame of a suitable burner till all the starch is carbonized. Complete the ignition in a muffle furnace at 550 ± 25°C for 3 hours. Cool in a desiccator and weigh. Repeat the process of ignition in the furnace, cooling and weighing at half hour intervals until the difference between the two successive weighings is less than one milligram. Note the lowest mass. Preserve the dish and contents for the determination of total ash excluding sodium chloride (*see* **6**).

5.2 Calculation

$$\text{Total ash (on dry basis) percent by mass} = \frac{(M_2 - M) \times 10\,000}{M_1\,(100 - X)}$$

where

M_2 = mass in g of the dish with the ash,

M = mass in g of the empty dish,

M_1 = mass in g of the sample, and

X = moisture content, percent by mass.

6. DETERMINATION OF TOTAL ASH EXCLUDING SODIUM CHLORIDE

6.1 Reagents

6.1.1 *Standard Silver Nitrate Solution* — 0·1 N.

6.1.2 *Standard Ammonium Thiocyanate Solution* — Standardized against standard silver nitrate solution.

6.1.3 *Dilute Nitric Acid (1 : 9)* — To 900 ml of water, add 100 ml of concentrated nitric acid (sp gr 1·42) and stir.

6.1.4 *Nitric Acid (4 : 1)* — To 100 ml of water, add 400 ml of concentrated nitric acid (sp gr 1·42). Bring the contents to boil until a colourless solution is obtained.

6.1.5 *Ferric Alum Indicator Solution* — Prepare a saturated solution of ferric alum in water and filter.

6.2 Procedure

6.2.1 Dissolve the ash (*see* **5**) in 25 ml of dilute nitric acid. Filter through Whatman filter paper No. 1 or its equivalent, collecting the filtrate in a 100-ml graduated flask, and wash the contents thoroughly with hot water. Make the volume to 100 ml.

6.2.2 To a 25-ml aliquot of the filtrate, add an excess of the standard silver nitrate solution (20 ml), stirring well to flocculate the precipitate of silver chloride. Filter and wash the precipitate thoroughly with water. To the combined filtrate, add 5 ml each of ferric alum indicator solution and concentrated nitric acid. Titrate the excess of silver nitrate with the standard ammonium thiocyanate solution to a stable light brown colour end point.

6.3 Calculation

$$\text{Sodium chloride (on dry basis), percent by mass} = \frac{5{\cdot}85\,(V_1N_1 - V_2N_2)\,100}{M_1\,(100 - X)} \times \frac{V_3}{V_4}$$

where

V_1 = volume in ml of the standard silver nitrate solution added initially,

N_1 = normality of the silver nitrate solution,

V_2 = volume in ml of the standard ammonium thiocyanate solution required for titrating the excess of silver nitrate,

N_2 = normality of the standard ammonium thiocyanate solution added,

M_1 = mass in g of the sample (M_1 under **5.2**),

X = moisture content, percent by mass (X under **5.2**),

V_3 = volume in ml to which the filtrate was made up, and

V_4 = volume in ml of the aliquot of the filtrate taken for the experiment.

6.3.1 To obtain total ash excluding sodium chloride (on dry basis), percent by mass, deduct the value obtained under **6.3** from that obtained under **5.2**.

7. DETERMINATION OF SULPHATED ASH

7.1 Reagent

7.1.1 *Concentrated Sulphuric Acid* — sp gr 1·84.

7.2 Procedure — Accurately weigh about 5 g of the sample into a 9 cm diameter platinum or silica dish. Add a few drops (about 1·5 ml) of concentrated sulphuric acid to the material in the dish. Gently heat the dish on a hot-plate until the material is well carbonized, and then increase the heat until the evolution of sulphuric acid fumes ceases. Ash the carbonized matter in a muffle furnace at 600 ± 20°C. Cool the ash and moisten it with a few drops of concentrated sulphuric acid, heat strongly on a hot-plate until sulphuric acid fumes cease to be evolved and finally ash in the muffle furnace at 600 ± 20°C for about 2 hours. Cool in a desiccator and weigh. Heat again in the muffle furnace for 30 minutes at 600 ± 20°C. Cool in a desiccator and weigh. Repeat the process of heating in the muffle furnace for 30 minutes, cooling and weighing till the difference between two successive weighings is less than 10 mg. Record the lowest mass.

7.3 Calculation

$$\text{Sulphated ash, percent by mass} = \frac{100\ M_1}{M_2}$$

where

M_1 = mass in g of the ash, and

M_2 = mass in g of the sample taken for the test.

8. DETERMINATION OF ACID INSOLUBLE ASH

8.1 Reagents

8.1.1 *Hydrochloric Acid* — Approximately 5 N, prepared from concentrated hydrochloric acid (*see* Analytical Reagent grade of IS : 265-1976*).

8.2 Procedure — Weigh accurately about 5 g of the material in a platinum, porcelain or silica dish and follow the procedure as given in **5.1**. To the ash obtained add 25 ml of hydrochloric acid and cover with a watch-glass and heat on a water-bath for 10 minutes. Allow to cool and filter the contents of the dish through Whatman filter paper No. 42 or its equivalent. Wash the filter paper with water until the washings are free from the acid. Return the filter and the residue to the dish. Keep it in an electric air-oven maintained at 105 to 110°C for about 3 hours. Ignite in

*Specification for hydrochloric acid (*second revision*).

a muffle furnace at 550 ± 20°C for 3 hours. Cool the dish in a desiccator and weigh. Repeat the process of igniting in the muffle furnace, cooling and weighing at half hour intervals until the difference between two successive weighings is less than one milligram. Note the lowest mass.

8.3 Calculation

$$\text{Acid insoluble ash (on dry basis), percent by mass} = \frac{10\,000\,(M_2 - M)}{M_1\,(100 - X)}$$

where

M_2 = mass in g of the dish with the acid insoluble ash;
M = mass in g of the empty dish;
M_1 = mass in g of the sample; and
X = moisture content, percent by mass.

9. DETERMINATION OF STARCH (ACID HYDROLYSIS METHOD)

9.0 General — Two chemical methods, namely, acid hydrolysis method and diastase hydrolysis method are generally used. Polarimeter is also employed to measure the optical activity of the starch solution, either after dispersion with hydrochloric acid or dispersion in calcium chloride solution. However, only the acid hydrolysis method is specified as it is the simplest, fairly reliable and accurate.

9.1 Reagents

9.1.1 *Ethyl Ether*

9.1.2 *Ethyl Alcohol* — 10 percent (*v/v*).

9.1.3 *Dilute Hydrochloric Acid* — 2·5 percent, prepared by mixing 20 ml of concentrated hydrochloric acid (sp gr 1·16) and 200 ml of water.

9.1.4 *Sodium Carbonate Solution* — 20 percent (*m/v*).

9.1.5 *Stock Solution of Dextrose* — Weigh accurately 10 g of anhydrous dextrose into a one-litre graduated flask and dissolve it in water. Add to this solution 2·5 g of benzoic acid, shake to dissolve benzoic acid and make up the volume to the mark with water. (This solution should not be used after 48 hours.)

9.1.6 *Standard Dextrose Solution* — Dilute a known aliquot of the stock solution of dextrose (**9.1.5**) with water containing 0·25 percent (*m/v*) of benzoic acid to such a concentration that more than 15 ml but less than 50 ml of it will be required to reduce all the copper in the Fehling's

solution taken for titration. Note the concentration of anhydrous dextrose in this solution as mg/100 ml (*see* Note). Prepare this solution fresh everyday.

NOTE — When 10 ml (*see also* **9.3.1**) of Fehling's solution are taken for titration, a standard dextrose solution containing 0·11 to 0·30 percent (*m/v*) of anhydrous dextrose is convenient for use.

9.1.7 *Methylene Blue Indicator Solution* — Dissolve 0·2 g of methylene blue in water and dilute to 100 ml.

9.1.8 *Fehling's Solution (Soxhlet Modification)* — Prepared by mixing immediately before use, equal volume of solution A and solution B which are prepared as follows:

a) *Solution A* — Dissolve 34·639 g of copper sulphate ($CuSO_4.5H_2O$) in water, add 0·5 ml of concentrated sulphuric acid of sp gr 1·84 and dilute to 500 ml in a graduated flask. Filter the solution through prepared asbestos.

b) *Solution B* — Dissolve 173 g of Rochelle salt (potassium sodium tartrate) ($KNaC_4H_4O_6.4H_2O$) and 50 g of sodium hydroxide in water, dilute to 500 ml in a graduated flask and allow the solution to stand for two days. Filter this solution through prepared asbestos.

9.1.9 *Standardization of Fehling's Solution* — Pour the standard dextrose solution (**9.1.6**) into a 50-ml burette (*see* Note 3 under **9.2.3**). Find the titre (that is, the volume of the standard dextrose solution required to reduce all the copper in 10 ml of Fehling's solution) corresponding to the concentration of the standard dextrose solution from Table 1. (If, for example, the standard dextrose solution contains 167·0 mg of anhydrous dextrose per 100 ml, the corresponding titre would be 30 ml.) Pipette 10 ml (*see also* **9.3.1**) of Fehling's solution into a 300-ml conical flask and run in from the burette almost the whole of the standard dextrose solution required to effect reduction of all the copper, so that not more than 1 ml will be required later to complete the titration. Heat the flask containing the mixture over a wire gauze. Gently boil the contents of the flask for 2 minutes. At the end of 2 minutes of boiling, add, without interrupting boiling, 1 ml of methylene blue indicator solution. While the contents of the flask continue to boil, begin to add standard dextrose solution (one or two drops at a time) from the burette till the blue colour of the indicator just disappears. [The titration should be completed within 1 minute, so that the contents of the flask boil altogether for 3 minutes without interruption (*see* Note 2 under **9.2.3**).] Note the titre. Multiply the titre (obtained by direct titration) by the number of milligrams of anhydrous dextrose in 1 ml of the standard dextrose solution to obtain the dextrose factor. Compare this factor with dextrose factor given in Table 1 and

determine the correction, if any, to be applied to the dextrose factors derived from Table 1.

Example:

Concentration of anhydrous dextrose in the standard dextrose solution as mg/100 ml	= 167·0
Titre obtained by direct titration	= 30·1 ml
Dextrose factor for 30·1 ml of the standard dextrose solution	= titre in ml X number of mg of anhydrous dextrose in 1 ml of the standard dextrose solution
	= 30·1 × 1·670
	= 50·2670
Dextrose factor for 30·1 ml of standard dextrose solution from Table 1 (calculated by interpolation)	= 50·1
Correction to be applied to the dextrose factors derived from Table 1	= 50·2670 — 50·11
	= + 0·1570

9.2 Procedure

9.2.1 *Preparation of the Solution* — Extract about 0·5 g of ground material, accurately weighed, with five 10-ml portions of ether on a filter paper that would retain completely the smallest starch granules. Evaporate the ether from the residue and wash with 150 ml of 10 percent ethyl alcohol. Carefully wash off the residue from the filter paper with 200 ml of cold water. Heat the undissolved residue with 220 ml of 2·5 percent dilute hydrochloric acid in a flask equipped with reflux condenser for 2½ hours. Cool and neutralize with sodium carbonate solution and transfer quantitatively to a 250-ml graduated flask and make up to volume.

9.2.2 *Incremental Method of Titration* — Pour the prepared solution(**9.2.1**) into a 50-ml burette (the same may be filtered if not clear) (*see* Note 3 under **9.2.3**). Pipette 10 ml of Fehling's solution into a 300 ml conical flask and run in from the burette 15 ml of the prepared solution. Without further dilution, heat the contents of the flask over a wire gauze, and boil. (After the liquid has been boiling for about 15 seconds, it will be possible to judge if almost all the copper is reduced by the bright red colour imparted to the boiling liquid by the suspended cuprous oxide). When it is judged that nearly all the copper is reduced, add 1 ml of the methylene blue indicator solution (*see* Note 1). Continue boiling the contents of the flask for one to two minutes from the commencement of

ebullition, and then add the prepared solution in small quantities (1 ml or less at a time), allowing the liquid to boil for about 10 seconds between successive additions, till the blue colour of the indicator just disappears (*see* Note 2 under **9.2.3**). In case there still appears to be much unreduced copper after the mixture of Fehling's solution with 15 ml of the prepared solution has been boiling for 15 seconds, add the prepared solution from the burette in larger increments (more than 1 ml at a time, according to judgement), and allow the mixture to boil for 15 seconds after each addition. Repeat the addition of the prepared solution at intervals of 15 seconds until it is considered unsafe to add a large increment of the prepared test solution. At this stage continue the boiling for an additional one to two minutes, add 1 ml of methylene blue indicator solution and complete the titration by adding the prepared solution in small quantities (less than 1 ml at time) (*see* Note 2).

NOTE 1 — It is advisable not to add the indicator until the end point has been nearly reached because the indicator retains its full colour until the end point is almost reached and thus gives no warning to the operator to go slowly.

NOTE 2 — When the operator has had a fair amount of experience with the method, a sufficiently accurate result may often be obtained by a single estimation by the incremental method of titration. For the utmost degree of accuracy of which the method is capable a second titration should be carried out by the standard method of titration (*see* **9.2.3**).

9.2.3 *Standard Method of Titration* — Pipette 10 ml of Fehling's solution into a 300-ml conical flask and run in from the burette almost the whole of the prepared solution required to effect reduction of all the copper (determined under **9.2.2**), so that if possible, not more than 1 ml will be required later to complete the titration. Gently boil the contents of the flask for 2 minutes. At the end of 2 minutes of boiling, add without interrupting the boiling, 1 ml of methylene blue indicator solution. While the contents of the flask continue to boil, begin to add the prepared solution (one or two drops at a time) from the burette till the blue colour of the indicator just disappears (*see* Note 1). [The titration should be completed within one minute, so that the contents of the flask boil altogether for 3 minutes without interruption (*see* Note 2).]

NOTE 1 — The indicator is so sensitive that it is possible to determine the end point within one drop of the prepared test solution in many cases. The complete decolourization of the methylene blue is usually indicated by the whole reaction liquid in which the cuprous oxide is continuously churned up becoming bright red or orange in colour. In case of doubt, the flame may be removed from the wire gauze for one or two seconds and the flask held against a sheet of white paper. (A folded paper, suitably fixed round the neck of the flask, is very convenient for this purpose as it may be left round the neck of the flask without risk of over-balancing it.) The top edge of the liquid would appear bluish if the indicator is not completely decolourized. It is inadvisable to interrupt the boiling as the indicator undergoes back oxidation rather rapidly when air is allowed free access into the flask, but there is no danger of this as long as a continuous stream of steam is issuing from the mouth of the flask.

NOTE 2 — It should be observed that with both incremental and standard methods of titration, the flask containing the reaction mixture is left on the wire gauze over the flame throughout the titration.

NOTE 3 — In adding sugar solution to the reaction mixture, the burette may be held in hand over the flask. The burette may be fitted with a small outlet tube bent twice at right angles, so that the body of the burette may be kept out of the steam while adding the sugar solution. Burettes with glass taps are unsuitable for this work, as the taps become heated by the steam and are liable to jam.

NOTE 4 — The dilution of the test solution (starch hydrolysate) should be such that its titre value lies between 15 and 50 ml. It is preferable to have a titre reading around 25 ml.

9.3 Calculation

9.3.1 Refer to Table 1 for the dextrose factor corresponding to the titre (determined as given under **9.2.3**) and apply the correction previously determined under **9.1.9**. Calculate the dextrose content of the prepared solution (*see* **9.2.1**) as follows:

$$\text{Milligrams of anhydrous dextrose present in 1 ml of the prepared solution} = m = \frac{\text{Dextrose factor}}{\text{Titre}}$$

Instead of using 10 ml of Fehling's solution, a 25-ml portion may also be substituted throughout the procedure (including standardization of Fehling's solution under **9.1.9**). In this case, the standard dextrose solution, used in standardizing the Fehling's solution, and the prepared solution of the material (*see* **9.2.1**) should contain 0·25 to 0·75 percent (*m/v*) of anhydrous dextrose, and Table 2 should be used for the calculations.

NOTE — Tables 1 and 2 show, for the standard method of titration, the values corresponding to integral millilitres of the sugar solutions, intermediate values being obtained by interpolation.

9.3.2 Starch (on dry basis), percent by mass $= \frac{9{\cdot}3\, mV}{M_1 (100 - M)}$

where

m = milligrams of anhydrous dextrose in one millilitre of the prepared solution of the material (**9.3.1**),

V = total volume in ml of the prepared solution (**9.2.1**),

M_1 = mass in g of the material used to prepare V ml of the solution, and

M = percentage of moisture, as determined in **4.**

TABLE 1 DEXTROSE FACTORS FOR 10 ml OF FEHLING'S SOLUTION

(*Clauses* 9.1.9 *and* 9.3.1)

TITRE	DEXTROSE FACTOR*	DEXTROSE CONTENT PER 100 ml OF SOLUTION
(1)	(2)	(3)
ml		mg
15	49·1	327
16	49·2	307
17	49·3	289
18	49·3	274
19	49·4	260
20	49·5	247·4
21	49·5	235·8
22	49·6	225·5
23	49·7	216·1
24	49·8	207·4
25	49·8	199·3
26	49·9	191·8
27	49·0	184·9
28	50·0	178·5
29	50·0	172·5
30	50·1	167·0
31	50·2	161·8
32	50·2	156·9
33	50·3	152·4
34	50·3	148·0
35	50·4	143·9
36	50·4	140·0
37	50·5	136·4
38	50·5	132·9
39	50·6	129·6
40	50·6	126·5
41	50·7	123·6
42	50·7	120·8
43	50·8	118·1
44	50·8	115·5
45	50·9	113·0
46	50·9	110·6
47	51·0	108·4
48	51·0	106·2
49	51·0	104·1
50	51·1	102·2

NOTE — If the value obtained is not comparable with the value given in the table then the standard sample of anhydrous dextrose shall be rechecked.

*Milligrams of anhydrous dextrose corresponding to 10 ml of Fehling's solution.

TABLE 2 DEXTROSE FACTORS FOR 25 ml OF FEHLING'S SOLUTION

(*Clause* 9.3.1)

TITRE	DEXTROSE FACTOR*	DEXTROSE CONTENT PER 100 ml OR SOLUTION
(1)	(2)	(3)
ml		mg
15	120·2	801
16	120·2	751
17	120·2	707
18	120·2	668
19	120·3	638
20	120·3	601·5
21	120·3	572·9
22	120·4	547·3
23	120·4	523·6
24	120·5	501·9
25	120·5	482·0
26	120·6	463·7
27	120·6	446·8
28	120·7	431·0
29	120·7	416·4
30	120·8	402·7
31	120·8	389·7
32	120·8	377·6
33	120·9	366·3
34	120·9	355·6
35	121·0	345·6
36	121·0	336·3
37	121·1	327·4
38	121·2	318·8
39	121·2	310·7
40	121·2	303·1
41	121·3	295·9
42	121·4	289·0
43	121·4	282·4
44	121·5	276·1
45	121·5	270·1
46	121·6	264·3
47	121·6	258·8
48	121·7	253·5
49	121·7	248·4
50	121·8	243·6

NOTE — If the value obtained is not comparable with the value given in the table then the standard sample of anhydrous dextrose shall be rechecked.

*Milligrams of anhydrous dextrose corresponding to 25 ml of Fehling's solution.

10. DETERMINATION OF PROTEIN (TITRIMETRIC METHOD)

10.1 Definition — Total protein, by the Kjeldahl method, is defined as the amount of nitrogen experimentally found and multiplied by an appropriate conversion factor (*see* **10.6.1** *and* **10.6.2**).

10.2 Principle — Destruction of organic matter by sulphuric acid in the presence of a compound catalyst, alkalization of the reaction products, distillation of the liberated ammonia and collection in a boric acid solution, followed by titration with a standard volumetric sulphuric acid solution.

10.3 Apparatus

10.3.1 *Kjeldahl Flask* — of suitable capacity, usually 500 to 800 ml, preferably with a ground-glass joint, and provided with a pear-shaped glass bulb fitting loosely in the top of the neck of the flask.

10.3.2 *Digestion Stand* — on which the Kjeldahl flask (*see* **10.3.1**) can be heated in an inclined position in such a way that heat is applied only to that part of the flask wall which is below the liquid level at all stages.

10.3.3 *Distillation or Steam Distillation Apparatus* — with a 200-ml graduated dropping funnel and an efficient splash head, the latter connecting the Kjeldahl flask (**10.3.1**) to the condenser.

10.3.4 *Burette* — 25 ml with 0·05 ml graduations, or 10 ml with 0·01 ml graduations.

10.3.5 *Mechanical Grinder or Mortar*

10.3.6 *Sieve* — with nominal mesh openings of 0·6 mm.

10.4 Reagents

10.4.1 *Concentrated Sulphuric Acid* — 96 percent by mass, nitrogenfree, sp gr 1·84 at 20°C.

10.4.2 *Sodium Hydroxide Solution* — 30 percent by mass, sp gr 1·33 at 20°C.

10.4.3 *Boric Acid* — Dissolve 20 g boric acid crystals in 1 litre distilled water.

10.4.4 *Compound Catalyst* — Consisting of 97 g potassium sulphate and 3 g anhydrous copper II sulphate.

10.4.5 *Standard Sulphuric Acid* — 0·02 N or 0·1 N.

10.4.6 *Indicator* — Prepare by mixing 2 parts by volume of a cold saturated solution of neutral methyl red in 50 percent ethyl alcohol with

1 part by volume of 0·025 percent solution of methylene blue in 50 percent ethyl alcohol. Store the indicator in a brown glass bottle.

10.5 Procedure

10.5.1 *Preparation of Sample* — Mix the sample thoroughly and rapidly by shaking or stirring with a spatula in the sample container. If the sample container is too small for this purpose, transfer the entire sample to another pre-dried container of a suitable size to facilitate mixing. In the case of glucose syrup, remove the surface layer (about 0·5 cm) before mixing. It may be necessary to grind the sample, in which case it shall all pass through the sieve (**10.3.2**) without leaving any residue.

10.5.2 *Test Portion* — Weigh, to the nearest 0·001 g, up to 10 g (mass M) of the prepared sample, according to the presumed nitrogen content, and transfer to the pre-dried Kjeldahl flask taking care that none of the product adheres to the inner wall of the neck of the flask. In the case of a viscous liquid or a product in paste form, the test portion may be weighed in a small glass container or on a sheet of aluminium, paper or plastics which does not yield nitrogen, or whose nitrogen content is known, and which is left in the flask. In the case of a container which yields nitrogen, this should be taken into account in the blank test (**10.5.4**).

10.5.3 *Digestion* — Add 10 g of the compound catalyst and using a suitable measuring cylinder, add an appropriate volume, in millilitres, of the concentrated sulphuric acid, calculated by the formula $20 + 4M$, in such a way that the acid rinses the inner wall of the neck of the flask. Mix the contents of the flask by swirling the flask gently until the mixture is free from lumps and the test portion is completely wetted. In order to avoid superheating, add a few glass beads or boiling chips. Insert the pear-shaped glass bulb (**10.3.1**) in the neck of the flask and place it in an inclined position on the digestion stand. Heat with care until the liquid in the flask boils gently. Continue to heat for 1 hour after the liquid becomes clear.

NOTE — In the case of digestion apparatus heated by gas, ensure that the flame does not extend beyond the part of the flask filled with liquid, in order to avoid loss of nitrogen.

10.5.4 *Distillation and Titration* — Allow the contents of the flask to cool and rinse the pear-shaped glass bulb and the inner-neck of the flask with a few millilitres of water, allowing the rinsings to run into the flask. Add, with care, between 50 and 200 ml of water (according to the apparatus used), whilst swirling the contents of the flask. Connect the flask to the distillation or steam distillation apparatus, previously freed from ammonia by steaming. Adjust the lower end of the condenser so that it just touches the bottom of a 500-ml conical flask containing a known volume (between 25 and 50 ml) of the boric acid solution and 3 to 5 drops of the titration indicator.

Render the digestion liquid alkaline by slowly adding through the graduated separating funnel, placed in the neck of the flask, between 150 and 200 ml of the sodium hydroxide solution ensuring that the stem of the funnel does not become empty. Mix well, then turn on the condenser water and start heating; the ammonia then begins to be carried over. The indicator contained in the flask turns immediately to its alkaline colour. During distillation, ensure that steam generation is steadily maintained. Distillation is complete when 200 ml of liquid have been collected in about 20 to 30 minutes. Turn off the heat and lower the conical flask. Allow the condenser to drip for a few minutes into the flask and rinse the tip of the condenser with water, collecting the rinsings in the conical flask. Titrate the contents of the flask the colour of which should be green with either 0·02 N or 0·1 N sulphuric acid solution using a 10 ml or 25 ml burette as appropriate, until the colour of the contents turns reddish violet. Carry out two determinations on the same prepared sample, and a blank determination on the reagents only. Express the result as the mean of the two determinations if the volumes of the sulphuric acid solution used do not differ by more than 0·1 ml, otherwise repeating the determinations on the same test sample.

10.6 Calculation

$$\text{Nitrogen content (on dry basis), percent by mass } (A) = \frac{(V - V_0) \times 140 \times N}{M\,(100 - X)}$$

where

V = volume in ml of sulphuric acid solution used in the determination,

V_0 = volume in ml of sulphuric acid solution used in the blank determination,

N = normality of the sulphuric acid solution,

M = mass in g of the test portion, and

X = moisture content, percent by mass (*see* **4**).

10.6.1 Protein content in starch derived from wheat (on dry basis), percent by mass = $A \times 5{\cdot}7$

10.6.2 Protein content in all other starches (on dry basis), percent by mass = $A \times 6{\cdot}25$

11. DETERMINATION OF SULPHUR DIOXIDE

11.0 Two methods are given for the determination of sulphur dioxide. Method I is included to serve as a reference method while Method II is to be used as a routine method.

11.1 Method I (Referee Method)

11.1.1 *Principle* — Sulphur dioxide is liberated from the test portion by boiling hydrochloric acid solution and removed by a stream of carbon dioxide. Sulphur dioxide is oxidised to sulphuric acid by bubbling the gas through a 3 percent solution of hydrogen peroxide and the sulphuric acid is titrated with 0·1 N sodium hydroxide solution.

11.1.2 *Apparatus*

11.1.2.1 *Sulphur dioxide apparatus* — as shown in Fig. 1, or equivalent. The apparatus illustrated comprises the following parts, connected together by means of ground-glass joints:

a) *Boiling flask* — round-bottomed, of 1 000 ml capacity, with three necks for connection to gas inlet tube, dropping funnel and reflux condenser;

b) *Gas inlet tube*;

c) *Dropping funnel* — 100 ml capacity;

d) *Delivery tube* — connecting the upper end of reflux condenser to neck of receiver; and

e) *Receiver* — comprising a 300-ml or 250-ml conical flask combined with absorption bulbs.

11.1.2.2 *Gas-washing bottle*

11.1.2.3 *Burette* — 10 ml with 0·02 graduations.

11.1.3 *Reagents*

11.1.3.1 *Carbon dioxide*

11.1.3.2 *Concentrated hydrochloric acid* — sp gr 1·18 at 20°C.

11.1.3.3 *Sodium carbonate solution* — Dissolve 7·5 g of anhydrous sodium carbonate in 100 ml of water.

11.1.3.4 *Hydrogen peroxide* — 3 percent solution, neutral. Dilute 10 ml of 30 percent hydrogen peroxide with 100 ml of water immediately before use.

11.1.3.5 *Standard sodium hydroxide solution* — 0·1 N.

11.1.3.6 *Bromophenol blue indicator* — Dissolve 0·04 g of bromophenol blue in 100 ml or a mixture of equal parts, by volume, of water and ethanol.

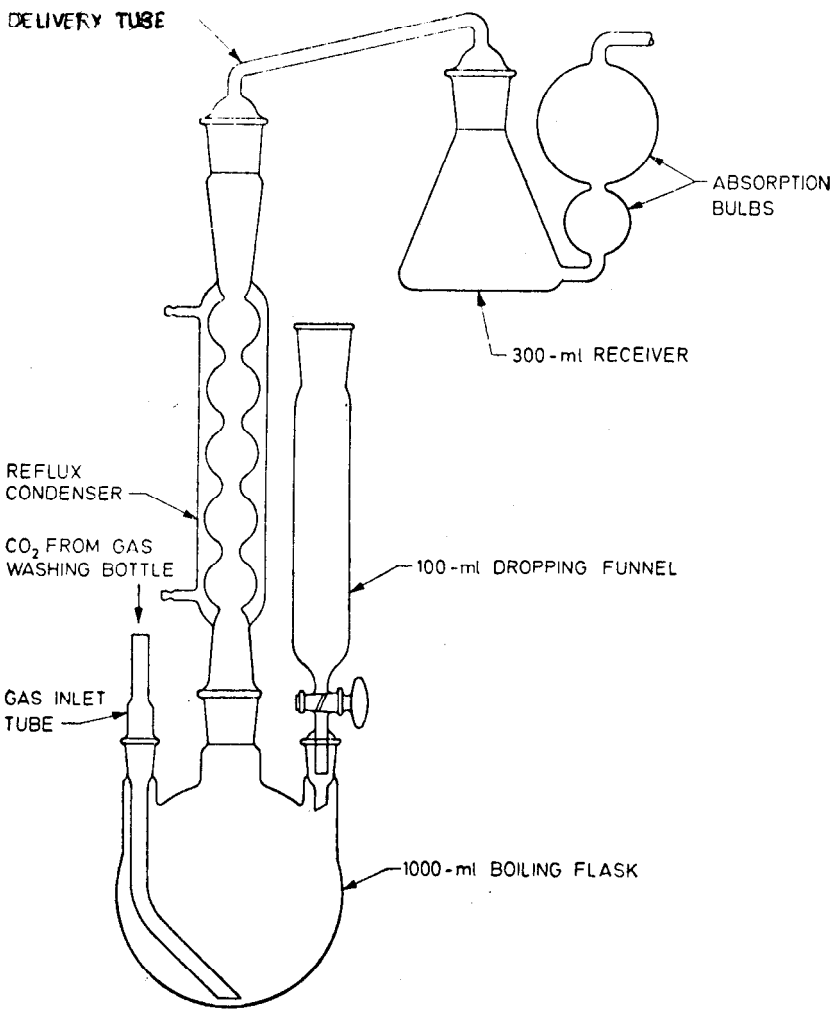

FIG. 1 SULPHUR DIOXIDE APPARATUS

11.1.4 *Preparation of Sample* — Before taking the test portion from the sample container, mix the sample thoroughly and rapidly by shaking or stirring with a spatula or spoon. If the original sample container is too small for this purpose, transfer the entire sample to a pre-dried larger container to facilitate mixing.

11.1.5 *Procedure* — Weigh the sample accurately to the nearest gram (according to the expected sulphur dioxide content of the sample), and dissolve or disperse it in water which may be double the amount of the sample. Pass carbon dioxide from a generator or cylinder through the gas-washing bottle containing sodium carbonate solution to remove chlorine, then into the gas inlet tube of the boiling flask. Introduce 25 ml of hydrogen peroxide into the receiver. Connect the apparatus and run into the boiling flask, by means of the dropping funnel, 200 ml water and 20 ml of concentrated hydrochloric acid. Boil the contents of the flask for about 10 minutes in a current of carbon dioxide. Transfer the solution or dispersion of the test portion to the boiling flask by means of the dropping funnel. Rinse the inner wall of the funnel with a few millilitres of water and run the rinsings into the flask. In case of samples of starch, place the dispersion into the funnel without boiling the hydrochloric acid solution. Pass carbon dioxide through the boiling flask for more than 20 minutes and begin heating only after having added the dispersion. Regulate the addition of the test portion and the gas flow rate through the apparatus to prevent drawback of hydrogen peroxide, inclusion of air, or burning of the test portion. Boil the mixture gently for 1 hour in a slow current of carbon dioxide. Stop the flow of water in the condenser just before the end of the distillation. When the delivery tube just above the receiving flask becomes hot, remove the receiving flask from the delivery tube immediately, add 3 ml of bromophenol blue indicator and titrate with the 0·1 N sodium hydroxide solution. Carry out two determinations on the same prepared sample.

11.1.5.1 Carry out a blank determination as in **11.1.5** on the reagents only but omitting the test portion. Take the mean of the two determinations as the result.

11.1.6 *Calculation*

$$\text{Sulphur dioxide content (on dry basis), mg/kg of the product} = \frac{(V - V_0) \times 3\,200\,000 \times N}{M_1 (100 - M)}$$

where

V = volume in ml of sodium hydroxide solution used for the test portion,

V_0 = volume in ml of sodium hydroxide solution used in the blank determination,

N = normality of sodium hydroxide solution,

M_1 = mass in g of test portion, and

M = percentage of moisture as determined in **4**.

11.2 Method II (Routine Method)

11.2.1 *Reagents*

11.2.1.1 *Sulphuric acid* — 6 N.

11.2.1.2 *Sodium hydroxide* — 1 N.

11.2.1.3 *Iodine solution* — 0·032 15 N.

11.2.1.4 *Starch solution indicator*

11.2.2 *Procedure* — Take 25 g of the sample and add about 25 ml distilled water, make homogenous liquid by warming. Cool the solution, and add 25 ml of 1 N sodium hydroxide solution. Keep it aside for half an hour. Then add 10 ml 6 N sulphuric acid and titrate against 0·031 25 N iodine solution using starch solution as an indicator. The end point gives dark blue permanent colour. Note down the titre reading. Calculate the mg/kg of sulphur dioxide.

11.2.3 *Calculation*

$$\text{Sulphur dioxide content (on dry basis), mg/kg of the product} = \frac{V \times 4\,000}{100 - M}$$

where

V = volume of 0·031 25 N iodine used for titration, and

M = percentage of moisture as determined in **4**.

12. DETERMINATION OF CRUDE FIBRE

12.1 Reagents

12.1.1 *Dilute Sulphuric Acid* — 1·25 percent (*m/v*), accurately prepared.

12.1.2 *Sodium Hydroxide Solution* — 1·25 percent (*m/v*), accurately prepared.

12.2 Procedure — Dry to constant mass about 5 g of the material in an electric air oven at 105 to 110°C. Weigh accurately about 2·5 g of the dried material into a thimble and extract for about 1 hour with petroleum ether, using a Soxhlet apparatus. Transfer the fat-free material to a 1-litre flask. Take 200 ml of dilute sulphuric acid in a beaker and bring to boil. Transfer fat-free material and immediately connect the flask with a reflux

water condenser and heat, so that the contents of the flask begin to boil within 1 minute. Rotate the flask frequently, taking care to keep the material from remaining on the sides of the flask out of contact with the acid. Continue boiling for exactly 30 minutes. Remove the flask and filter through fine linen or silk cloth (75 micron) held in a funnel, and wash with boiling water until the washings are no longer acid to litmus.

Bring to boil some quantity of sodium hydroxide solution under a reflux condenser. Wash the residue on the linen into the flask with 200 ml of the boiling sodium hydroxide solution. Immediately connect the flask with the reflux condenser and boil for exactly 30 minutes. Remove the flask and immediately filter through the filtering cloth. Thoroughly wash the residue with boiling water and transfer to a Gooch crucible prepared with a thin but compact layer of ignited asbestos. Wash the residue thoroughly first with hot water and then with about 15 ml of ethyl alcohol, 95 percent by volume. Dry the Gooch crucible and contents at 105 to 110°C in an air-oven to constant mass. Cool and weigh. Incinerate the contents of the Gooch crucible in an electric muffle furnace at 600 $\pm$ 20°C until all the carbonaceous matter is burnt. Cool the Gooch crucible containing the ash in a desiccator and weigh.

NOTE — If the fat is less than 1 percent the test need not be performed.

12.3 Calculation

$$\text{Crude fibre (on dry basis), percent by mass} = \frac{100\ (M_1 - M_2)}{M}$$

where

M_1 = mass in g of Gooch crucible and contents before ashing,

M_2 = mass in g of Gooch crucible containing asbestos and ash, and

M = mass in g of the dried material taken for the test.

13. DETERMINATION OF *p*H OF AQUEOUS EXTRACT (ELECTROMETRIC METHOD)

13.1 Apparatus

13.1.1 *Electrodes and Potentiometric Equipment* — Calibrated against known buffer solution.

13.1.2 *Conical Flask*

13.2 Reagents

13.2.1 *Buffer Solutions* — of known *pH* values of 4·5 to 7.

13.3 Procedure — Place 10 g of the test sample in a dry conical flask and add 100 ml of cool, recently boiled distilled water. Agitate the flask until an even suspension, free from lumps, is obtained. Allow suspension to stand at 25°C for 30 minutes, agitating continuously or intermittently in such a manner as to keep the starch particles in suspension. Let it stand for 10 more minutes. Decant the supernatant liquid into the electrode vessel and immediately determine *pH* using a potentiometer and electrodes which have been calibrated against known buffer solutions.

14. DETERMINATION OF FREE ACIDITY

14.1 Reagents

14.1.1 *Standard Sodium Hydroxide Solution* — approximately 0·1 N.

14.1.2 *Phenolphthalein Indicator Solution* — Dissolve phenolphthalein in rectified spirit to yield a 1 percent (*m/v*) solution.

14.1.3 *Neutral Distilled Water* — To 100 ml of freshly boiled and cooled distilled water, add a few drops of phenolphthalein indicator solution and then add, drop by drop, approximately 0·01 N sodium hydroxide solution carefully from a burette until a permanent faint pink colour is produced.

14.2 Procedure — Wash a 250-ml beaker and a glass rod with adequate quantity of neutral distilled water. Weigh accurately about 10 g of the test sample and transfer it to the beaker. Add about 100 ml of neutral distilled water and 2 drops of phenolphthalein indicator solution. Stir the contents well with the glass rod taking care that no drops splash out. Titrate the contents with standard sodium hydroxide solution. Take the end point to have been reached when the colour of the solution under titration changes to permanent pink. Note the amount of alkali required to reach the end point.

14.3 Calculation

$$\text{Free acidity expressed as ml of 0·1 N sodium hydroxide solution required for 100 g of the sample (on oven-dry basis)} = \frac{100\,000\,VN}{M_1\,(100 - M)}$$

where

V = volume in ml of standard sodium hydroxide solution used up in the titration,

N = normality of sodium hydroxide solution,

M_1 = mass in g of the sample taken, and

M = moisture content, percent by mass, of the sample as determined in **4**.

15. DETERMINATION OF ALCOHOLIC ACIDITY

15.1 Reagent

15.1.1 *Neutral Ethyl Alcohol* — 90 percent (*v/v*).

15.1.2 *Standard Sodium Hydroxide Solution* — approximately 0·05 N.

15.1.3 *Phenolphthalein Indicator Solution* — Dissolve 0·1 g of phenolphthalein in 100 ml of 60 percent (*v/v*) rectified spirit.

15.2 Procedure — Weigh 5 g of sample into a conical stoppered flask and add 50 ml of neutral ethyl alcohol. Stopper, shake and allow to stand for 24 hours with occasional shaking. Filter the alcoholic extract through a dry filter paper. Titrate 10 ml of the combined alcoholic extract against standard sodium hydroxide solution using phenolphthalein as indicator. Calculate the percentage of alcoholic acidity as sulphuric acid.

15.3 Calculation

$$\text{Alcoholic acidity (as } H_2SO_4 \text{), with 90 percent alcohol, percent by mass} = \frac{24{\cdot}52\ A\ N}{M}$$

where

A = volume in ml of standard sodium hydroxide solution used in titration,

N = normality of standard sodium hydroxide solution, and

M = mass in g of the material taken for the test.

16. TEST FOR PRESENCE OF HYDROCYANIC ACID

16.1 Reagents

16.1.1 *Picric Acid Solution* — one percent (*m/v*), aqueous.

16.1.2 *Sodium Carbonate Solution* — 10 percent (*m/v*).

16.1.3 *Sodium Picrate Paper* — Prepare strips of filter paper. Dip them in the picric acid solution and dry. Then dip these in the sodium carbonate solution and again dry. Preserve these strips in a stoppered bottle.

16.1.4 *Chloroform*

16.2 Procedure — Take a small quantity of the material. Place the material in a test tube. Insert in the test tube, a moistened sodium picrate paper, taking care that it does not come in contact with the material. Add a few drops of chloroform and stopper the test tube tightly. Note the colour of the sodium picrate paper. If hydrocyanic acid is present, the colour changes to orange.

NOTE — This test is a delicate one and the rapidity of the colour change depends on the quantity of free hydrocyanic acid present in the material.

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